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Desensitizing Effect of Intra-Tumoral GDF-15 on Immunotherapy in Patients With Advanced Non-Small Cell Lung Cancer

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Keywords: cachexia | GDF-15 | non-small cell lung cancer | PD-1/PD-L1 inhibitors | survival

ABSTRACT

Background: Serum growth/differentiation factor 15 (GDF-15) suppresses anti-tumor immunity and predicts prognosis in several malignancies. Elevated GDF-15 levels are linked to cancer cachexia, characterized by weight loss and systemic inflammation, adversely affecting patient outcomes and therapy response. However, serum GDF-15 is not always derived from tumor tissues but also from multiple organs. Therefore, we evaluated whether intra-tumoral GDF-15 could be used as a biomarker for immunotherapy and its potential association with cancer cachexia.

Method: We retrospectively evaluated patients with advanced non-small cell lung cancer (NSCLC) who underwent treatment with programmed cell death-1 (PD-1)/programmed cell death-ligand 1 (PD-L1) inhibitors at the Shizuoka Cancer Center between 2017 and 2021. Patients with histologically confirmed NSCLC (stage III–IV or postoperative recurrence) who had undergone biopsy or surgery within 6 months prior to initiating immunotherapy were included. Expression of tumor-derived GDF-15 was evaluated using immunohistochemical staining of archival biopsy and surgical specimens. We analyzed the correlation between intra-tumoral GDF-15 expression and the incidence of cancer cachexia, as well as its impact on progression-free survival (PFS) and overall survival (OS).

Result: In 6 of 35 cases, tumor cells highly expressed GDF-15. Patients with high intra-tumoral GDF-15 expression had a higher incidence of cancer cachexia (100% vs. 41.4%, $p < 0.05$), shorter PFS (3.4 vs. 13.4 months, $p < 0.05$), and shorter OS (9.5 vs. 26.5 months, $p < 0.05$) than those with low intra-tumoral GDF-15 expression.

Conclusion: Intra-tumoral GDF-15 expression may predict the presence of cancer cachexia and the efficacy of PD-1/PD-L1 inhibitors in patients with advanced non-small cell lung cancer.

1 | Background

Growth/differentiation factor 15 (GDF-15) belongs to the transforming growth factor-β (TGF-β) superfamily and is physiologically absent or poorly expressed in most human tissues, except for the placenta and prostate [1, 2]. Previous studies have reported that higher plasma GDF-15 levels are associated with poorer outcomes and may serve as a useful biomarker in various malignancies, including non-small cell lung cancer (NSCLC) [3], colorectal cancer [4, 5], gastric cancer [6, 7], hepatocellular cancer [8], ovarian cancer [9], melanoma [10], breast cancer [11], myeloma [12, 13], and oral cancer [14]. However, plasma GDF-15 levels are elevated not only in the presence of cancer but also in cases of myocardial ischemia, inflammation, metabolic syndrome, stress, and aging. Additionally, the site of endogenous plasma GDF-15 secretion in patients with cancer remains unclear [15].

GDF-15 is primarily associated with cancer immunosurveillance. It is considered to facilitate tumor immune evasion by suppressing the activation of naïve $CD4^+$ and $CD8^+$ T cells by dendritic cells and the activation of cytotoxic T lymphocytes [16]. A previous study has reported that the depletion of intratumoral GDF-15 expression increases T-cell infiltration into the tumor, improves the immune response, and prolongs survival [15]. Furthermore, in recent years, strategies to inhibit the GDF-15 pathway have gained attention as a promising method to improve cancer cachexia-related symptoms such as decreased appetite and unintended weight loss. Cancer cachexia is a significant factor that worsens the prognosis in many patients, and inhibiting GDF-15 to alleviate these symptoms may potentially improve patients' quality of life and enhance the efficacy of subsequent treatments [17].

PD-1/PD-L1 inhibitors are immune checkpoint inhibitors that target the interaction between the T-cell co-inhibitory receptor programmed cell death 1 (PD-1) and one of its ligands, programmed cell death ligand 1 (PD-L1), and have recently been demonstrated to be effective in non-small cell lung cancer [18, 19]. However, PD-1 inhibitors are only effective in a limited population of NSCLC patients, and the response rate is approximately 50% even among patients with favorable intra-tumoral PD-L1 levels of ≥ 50% [18]. Therefore, new biomarkers beyond PD-L1 expression in tumor tissues are needed. Several reports have indicated that cancer cachexia significantly diminishes the efficacy of immunotherapy. Notably, this effect is not solely due to a decline in Eastern Cooperative Oncology Group (ECOG) performance status (PS); even patients with good PS who meet the criteria for cachexia experience reduced benefits from immunotherapy. This study investigated the potential of intratumoral GDF-15 expression as a predictive marker for outcomes with PD-1/PD-L1 inhibitor monotherapy.

2 | Methods

2.1 | Patients

We retrospectively evaluated NSCLC patients who underwent PD-1/PD-L1 inhibitor monotherapy at the Shizuoka Cancer Center between March 2017 and November 2021. Ethical approval for this study was obtained from the Shizuoka Cancer Center Ethics Committee. The eligibility criteria included: (1) histologically proven stage III–IV cancer according to TNM staging (American Joint Committee on Cancer, Eighth edition) or postoperative recurrence; (2) availability of sufficient stainable histopathology specimens; and (3) no history of immunotherapy. The exclusion criteria were as follows: (1) an interval > 6 months between biopsy and the initiation of PD-1/PD-L1 inhibitor therapy; (2) the presence of driver oncogenes (EGFR/ALK/ROS1); (3) Eastern Cooperative Oncology Group (ECOG) performance status (PS) ≥ 2; (4) unavailability of data regarding PD-L1 status; (5) weight change of unknown cause within 6 months before receiving PD-1/PD-L1 inhibitors; and (6) biopsy performed with histopathology specimens obtained using transbronchial lung biopsy (TBB). Biopsy performed with histopathological specimens obtained using TBB were excluded because this method has been demonstrated to have the lowest tumor volume on pathological examination and the lowest correlation with surgical specimens regarding PD-L1 expression [20, 21].

2.2 | Definition of Cachexia

Cancer cachexia was defined based on the international consensus criteria by Fearon et al., which include unintentional weight loss > 5% over 6 months, or > 2% in individuals already showing depletion according to body mass index (BMI < 20 kg/m^2) [22].

2.3 | Human-Derived Cell Cultures as Positive and Negative Controls

Paraffin-embedded tissues comprising MKN45 cells that endogenously expressed GDF15 and A549 cells that did not express GDF15 were prepared as positive and negative controls, respectively (Figure 1). Cells were seeded in 10-cm diameter petri dishes, cultured to approximately 80% confluency, washed twice with Dulbecco's Phosphate-Buffered Saline (DPBS), submerged in 3 mL of fresh DPBS, and collected as a cell suspension using a cell scraper (Sumitomo Bakelite Co. Ltd., MS-93170). The cell suspension was transferred into a fresh 1.5 mL Eppendorf tube, the supernatant was removed following centrifugation (140 × g, 5 min, room temperature), and cells were re-suspended in a gelatinization reagent (iPGell [23], GenoStaff, GSPG20-1) and allowed to gelatinize at room temperature. Cells were fixed in 10% neutral-buffered formalin overnight, and paraffin-embedded tissues were prepared using an automated paraffin displacement device (TISSUE Tek VIP 6 AI, Japan).

2.4 | Immunohistochemical Analysis

Tissue sections of archival formalin-fixed paraffin-embedded (FFPE) blocks of biopsy or surgical specimens were used for immunohistochemical staining. The sections were incubated with primary antibodies against GDF15 (SIGMA-ALDRICH[HPA011191]) in a 1:100 dilution for 60 min, followed by visualization with the Leica Bond Polymer Refine Detection

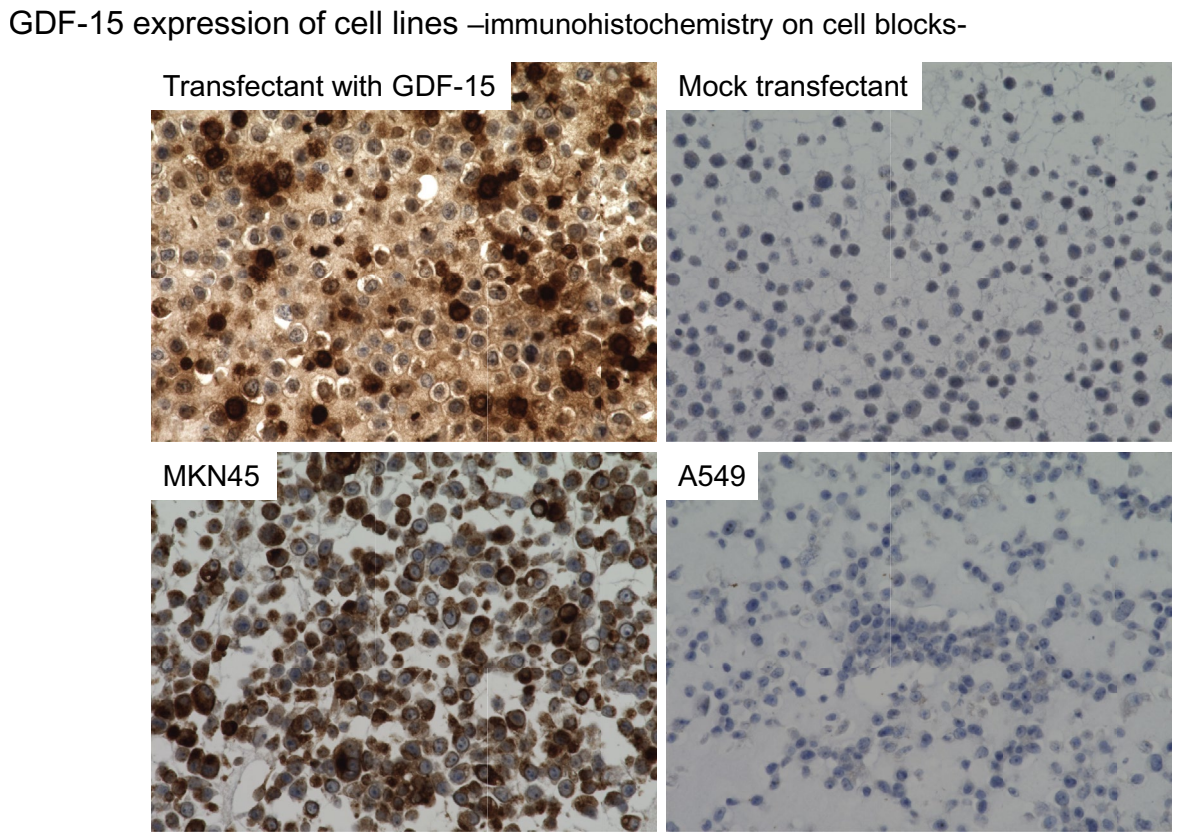


FIGURE 1 | The upper panel shows Chinese hamster cells stained with antibodies against GDF-15. ExpiCHO-S were transfected with the GDF15 expression vector as a positive control and mock transfectant with an empty expression vector as a negative control. The lower panel shows GDF15 expression in human-derived cells, where MKN45, which endogenously expresses GDF-15, was used as a positive control, and A549, which does not express GDF-15, was not stained, as in the negative control.

kit (DS9800). All slides were processed on the Autostainer Bond-III platform (Leica Biosystems, Wetzlar, Germany). Antigen retrieval was performed by Bond Epitope Retrieval Solution 2 [ER2] (pH 9.0) for 20 min at 100°C. The specimens were then counterstained with hematoxylin and coverslipped. We used cell blocks of human gastric cancer cell line, MKN45, and ExpiCHO-S cells transfected with human GDF-15 expression vector (Thermofisher) as positive control, and those of human lung cancer cell line, A549, and Mock transfectant of ExpiCHO-S cells as negative control (Figure 1).

2.5 | Scoring System for Assessing GDF-15 Expression

Immunoreactivity was evaluated using a semiquantitative scoring method that was based on the determination of the staining intensity and the proportion of positive tumor cells by a pathologist. The staining intensity was classified as follows: score 0 if there was no staining, score 1 if staining was weaker than the positive control cells, and score 2 if staining was as strong as the control cells (Figure 2A). The proportion of positive cells was classified into four categories: 0% (score 0), 1–19% (score 1), 20%–49% (score 2), 50%–79% (score 3), ≥80% (score 4). The overall score was calculated by multiplying each score, and then the grade of GDF-15 expression was categorized as high (≥ score 4) and low (< score 4) grade (Figure 2B,C).

2.6 | Statistical Analyses

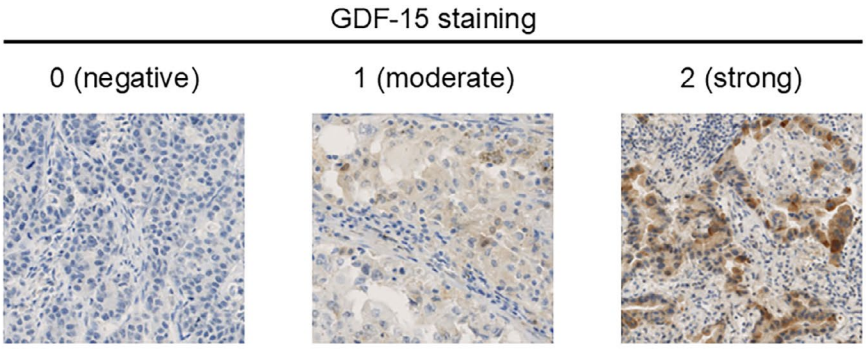
Statistical analyses were conducted using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan). Patient characteristics were presented as medians ranges. Comparisons between the high and low GDF-15 expression groups were made using the chi-square test or Fisher's exact test, as appropriate, and the Mann–Whitney U test was used for continuous variables. Progression-free survival (PFS) was measured from the initiation of PD-1/PD-L1 inhibitor therapy until disease progression or death, whichever came first. Overall survival (OS) was measured from the beginning of treatment to the time of death from any cause. PFS and OS were calculated using the Kaplan–Meier method, and comparisons between groups were performed using the log-rank test. Additionally, cox proportional hazards models, both univariate and multivariate, were utilized to assess hazard ratios and 95% confidence intervals for progression-free and overall survival. Statistical significance was defined as a two-sided p-value <0.05.

3 | Results

3.1 | Patient Characteristics

A flow diagram detailing patient recruitment for the study is shown in Figure 3. Of the 285 consecutive patients who underwent treatment with PD-1/PD-L1 inhibitors for advanced

(A)



(B)

GDF15 intensity of immunoreactivity staining	GDF15 stained positive cells				
	0	1	2	3	4
0	0	0	0	0	0
1	0	1	2	3	4
2	0	2	4	6	8

(C)

GDF-15 Classification	Score	n
Low N=29	0	12
	1	5
	2	12
	3	0
High N=6	4	2
	6	2
	8	2

FIGURE 2 | Representative images of the intensity of GDF-15 expression in immunohistochemistry graded as strong (2+), moderate (1+), and negative (0). (B) Scoring system for GDF-15 expression combined of the staining intensity and proportion of positive cells. (C) Classification of GDF-15 expression into high or low grade using the scoring system.

NSCLC, 35 were included in this study. We excluded 250 patients for the following reasons: 45 patients had an interval ≥ 6 months between biopsy and initiation of PD-1/PD-L1 inhibitor therapy, 47 had driver mutations, 28 had an ECOG PS ≥ 2, 85 lacked data regarding their PD-L1 status, 34 had unknown or unmeasured weight loss during the 6 months, and 11 underwent tissue biopsy using TBLB.

Characteristics of the 35 included patients are presented in Table 1. The median patient age was 70 years (range: 49–84). All patients had an ECOG PS score of 0 or 1. Most patients were men and had a non-squamous tumor histology (Table 1). Sixteen patients (45.7%) underwent needle biopsy, 13 (37.1%) underwent Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA), four patients (11.4%) underwent surgery, and two (5.7%) underwent cell block. Twenty-five patients (71.4%) underwent PD-1/PD-L1 inhibitor monotherapy as first-line treatment. Pretreatment PD-L1 TPS was measured for all patients; 29 patients (82.9%) had a TPS ≥ 50%. Eighteen patients (51.8%) were diagnosed with cancer-related cachexia at baseline. The mean level of albumin was 3.6 ± 0.7(g/dL, SD), and the median C reactive protein (CRP) level was 1.5 (range: 0.0–13.5) mg/dL.

3.2 | GDF-15 Expression in Tumor Tissue

Figure 4 shows a representative GDF-15 immunostaining image of a clinical samples collected from patients with adenocarcinoma. A trained pathologist classified parts of the tumor tissue as strong (2+) for GDF-15 expression, while neighboring areas were classified as negative (0). Therefore, GDF-15 protein expression in tumor tissue was not homogeneous.

Based on the scoring system, we divided the patients into the high ($n = 6$) and low ($n = 29$) GDF-15 groups. The high GDF-15 group had a higher incidence of cancer cachexia (100% vs. 41.4%, $p < 0.05$) and higher serum CRP levels (0.32 vs. 2.14 mg/dL, $p < 0.05$) than the low GDF-15 group (Table 1).

3.3 | Treatment Response

Herein, the overall response rate (ORR) was 42.9%. Patients with TPS ≥ 50% had a higher ORR than those with TPS < 50% (51.7% vs. 0%, $p = 0.027$). However, no significant difference in ORR was observed between the high and low GDF-15 groups (16.7% vs. 48.3%, $p = 0.20$).

3.4 | Impact of GDF-15 on Progression-Free Survival

Of the 35 patients, 25 (71.4%) showed tumor progression until the cut-off date (August 19, 2021). After a median follow-up of 9.9 months (95% CI, 3.7–16.3 months), patients with high GDF-15 levels demonstrated a shorter PFS than those with low GDF-15 levels (3.4 vs. 13.4 months, $p < 0.05$, Figure 5A). No statistically

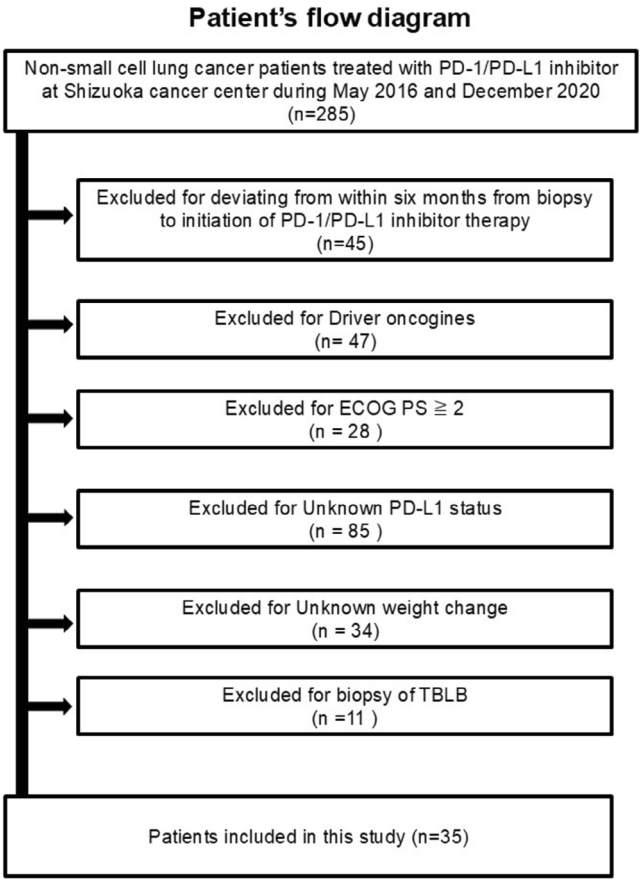


FIGURE 3 | Flow diagram of patient enrollment. In total, 285 patients were enrolled in this study. However, 250 patients were excluded for the following reasons: 45 patients were excluded for having an interval > 6 months between biopsy and the initiation of PD-1/PD-L1 inhibitor therapy, 47 had any kind of driver oncogenes, 28 had ECOG-PS ≧ 2, 85 did not have data regarding PD-L1 status, 34 did not have their weight changes examined over a 6-month duration, and 11 had only small tissue biopsied using TBLB. CT, computed tomography; PD-1, programmed cell death 1; PD-L1, programmed death-ligand 1; PS, performance status; TBLB, transbronchial lung biopsy.

significant difference in PFS was observed between patients with high (TPS ≥ 50%) and low PD-L1 expression (12.4 vs. 2.5 months, $p = 0.31$).

3.5 | Impact of GDF-15 on Overall Survival

Among the 35 patients, 21 (60.0%) died before the cut-off date. After a median follow-up of 19.7 months (95% CI, 14.3–33.3 months), patients with high GDF-15 levels demonstrated a shorter OS than those with low GDF-15 levels (9.5 vs. 26.5 months, $p < 0.05$, Figure 5B). No significant difference in OS was observed between patients with high (TPS ≥ 50%) and low PD-L1 expression (24.2 vs. 13.6 months, $p = 0.23$).

4 | Discussion

To our knowledge, only a few studies have shown that GDF-15 expression in tumors is heterogeneous and have verified the therapeutic effects of PD-1/PD-L1 inhibitors by assessing the expression of tissue GDF-15. Herein, we observed that patients with high GDF-15 expression in tumor tissues tended to exhibit cachexia and had significantly lower PFS and OS than those with low GDF-15 expression. These results indicated that GDF-15 may be a predictor of the efficacy of PD-1/PD-L1 inhibitors.

Although several studies have suggested that an elevated serum GDF-15 level is a poor prognostic factor for solid tumors [3–14], few studies have reported that high expression of GDF-15 in tumor tissues is a poor prognostic factor. Moreover, serum GDF-15 levels have been reported to be not necessarily elevated due to the tumor but also due to multiple factors, including myocardial injury and inflammation, as well as stress and aging [1–3]. One study reported no association between GDF-15 expression in tumor tissues and serum GDF-15 levels [24]. Therefore, serum GDF-15 may be secreted from other organs and not tumors [25]. Additionally, an in vivo study recently reported that serum GDF-15 binds to GFRAL, a receptor expressed in the hypothalamus [26], causing cachexia in the central nervous system [27]. No study has reported that GDF-15 expression in tumor tissues is associated with cachexia; however, it remains a possibility. Herein, patients with high tumor GDF-15 expression had a higher incidence of cachexia than those with low tumor GDF-15 expression. Considering that cachexia has already been reported to be a negative predictor of the therapeutic efficacy of PD-1/PD-L1 inhibitors in several studies, it is possible that high GDF-15 expression in tumor tissues causes cachexia, reducing the therapeutic efficacy of PD-1/PD-L1 inhibitors.

GDF-15 is not naturally expressed in human tissues, except in tumors, placenta, and fetal membranes [25]. The reason for the high GDF-15 expression in placental and fetal membrane tissues has been hypothesized to be related to fetal–maternal immune tolerance [25, 28]. Furthermore, serum GDF-15 derived from the placenta tends to be elevated in early pregnancy, and a retrospective study reported that serum GDF-15 levels tended to be low in pregnant women who experienced miscarriages [29, 30]. These studies suggest that GDF-15-expressing tissues may avoid attack by maternal immunity and that this system may play an essential role in protecting the fetus. This GDF-15-mediated immune tolerance may also occur in tumors, and the high expression of GDF-15 in tumors may reduce the therapeutic efficacy of PD-1/PD-L1 inhibitors. Several studies have reported that increased tissue GDF-15 expression is correlated with shorter recurrence time and tumor growth in vitro and in vivo [15, 31, 32]. The most significant factor leading to this observation is that the high expression of GDF-15 in tumors inhibits the activation of T cells involved in tumor immunity and suppresses T cell infiltration into tumors [15]. Previous studies have shown that elevated levels of CD8+ tumor-infiltrating lymphocytes in tumor tissues are essential markers for predicting the therapeutic effects of PD-1/PD-L1 inhibitors [33–35]. Therefore, high expression of GDF-15 in tumor tissues may reduce the therapeutic efficacy of PD-1/PD-L1 inhibitors by interfering with T-cell infiltration into tumors. Although several studies have reported that serum GDF-15 levels can predict the efficacy of anticancer medications, including cytotoxic regimens and immunotherapy [9, 36, 37], these predictive powers are limited. GDF-15

TABLE 1 | Patients' characteristics.

Characteristics		Total ($n=35$)	GDF-15		p
			High ($n=6$)	Low ($n=29$)	
Age median (range)		70 (49–84)	75 (66–78)	69 (49–84)	$p=0.08$
Gender (%)	Men	29 (82.9%)	5 (83.3%)	24 (82.8%)	NS
	Women	6 (17.1%)	1 (16.7%)	5 (17.2%)	
ECOG-PS (%)	0	9 (25.7%)	0	9 (31.0%)	NS
	1	26 (74.3%)	6 (100%)	20 (69.0%)	
Histology (%)	Squamous	6 (17.1%)	1 (16.7%)	5 (17.2%)	NS
	Non-squamous	29 (74.3%)	5 (83.3%)	24 (82.8%)	
Biopsy	Needle biopsy	16 (45.7%)	2 (33.3%)	14 (48.3%)	NS
	EBUS-TBNA	13 (37.1%)	2 (33.3%)	11 (37.9%)	
	Surgical	4 (11.4%)	0	4 (13.8%)	
	Cell block	2 (5.7%)	2 (33.3%)	0	
Duration from biopsy to ICI administration (days)		47 (16–158)	38.5 (21–90)	52 (16–158)	NS
Stage (%)	Postoperative recurrence	5 (14.3%)	0	5 (17.2%)	NS
	IV	30 (85.7%)	6 (100%)	24 (82.8%)	
PD-L1 tumor proportion score (%)	≥ 50%	29 (82.9%)	5 (83.3%)	24 (82.8%)	NS
	< 50%	6 (17.1%)	1 (16.7%)	5 (17.2%)	
Treatment line (%)	1	25 (71.4%)	5 (83.3%)	20 (69.0%)	NS
	≥ 2	10 (28.6%)	1 (16.7%)	9 (31.0%)	
Initial PD-1/PD-L1 inhibitor (%)	Nivolumab	1 (2.9%)	0	1 (3.4%)	NS
	Pembrolizumab	32 (91.4%)	5 (83.3%)	27 (93.2%)	
	Atezolizumab	2 (5.7%)	1 (16.7%)	1 (3.4%)	
Pretreatment cachexia[a] (%)		18 (51.4%)	6 (100%)	12 (41.4)	$p=0.02$
BMI (kg/m^2, mean ± SD)	≥ 20	22 (62.9%)	5 (83.3%)	17 (58.6%)	NS
	< 20	13 (37.1%)	1 (16.7%)	12 (41.4%)	
Albumin (g/dL, mean ± SD)		3.6 ± 0.7	3.8 ± 0.5	3.5 ± 0.7	NS
CRP (mg/dL, range)		1.5 (0.0–13.5)	0.32 (0.0–1.3)	2.14 (0.0–13.5)	$p=0.02$
GNRI		97.5 (66.6–123.6)	108.2 (93.5–113.7)	97.4 (66.6–123.6)	NS

Abbreviations: EBUS-TBNA, Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration; ECOG PS, Eastern Cooperative Oncology Group Performance Status; GNRI, Geriatric Nutritional Risk Index.
[a]Cancer cachexia was diagnosed according to the international consensus criteria proposed by Fearon et al., which include unintentional weight loss > 5% over 6 months, or > 2% in individuals already showing depletion according to body mass index (BMI < 20 kg/m^2).

expression in tumor tissues may be more tumor-specific and able to more accurately predict the efficacy of PD-1/PD-L1 inhibitors because various factors can alter serum GDF-15 levels. Further studies are required to confirm these hypotheses.

Recently, several novel therapeutic agents targeting the GDF-15–GFRAL axis have been developed. Most are designed to reduce anorexia and cancer cachexia (Ponsegromab, NCT05546476); however, few agents have been developed with the ambitious goal of sensitizing patients to immunotherapy [38]. CTL-002, a GDF-15 neutralizing antibody, is currently being assessed in clinical trials as a sensitizer for PD-1/PD-L1 inhibitors via the inhibition of tumor-derived GDF-15 [39]. A study evaluating the use of nivolumab in combination with CTL-002 for tumors with acquired resistance to PD-1/PD-L1 inhibitors reported instances of tumor shrinkage [40]. These findings suggest the potential therapeutic benefits of CTL-002.

However, given that GDF-15 is secreted from sources other than tumors and that there is no correlation between GDF-15

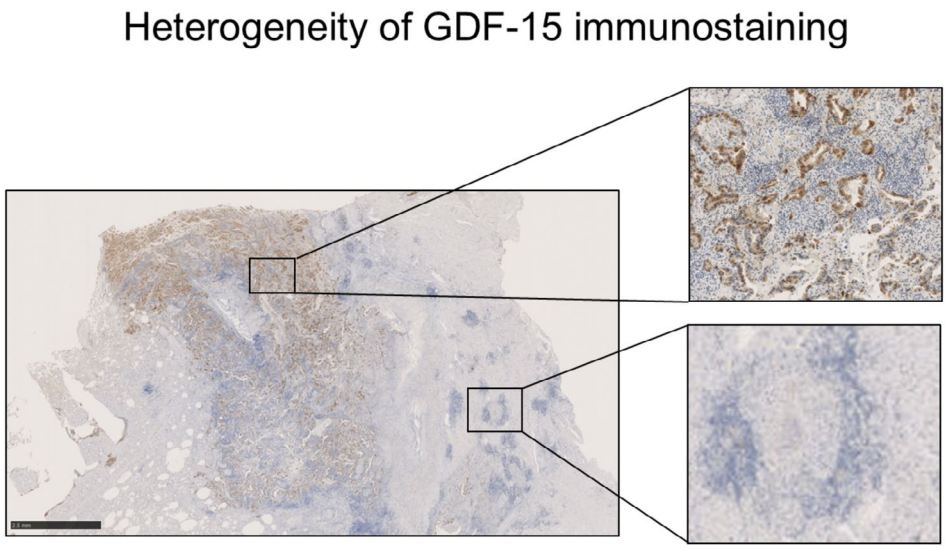


FIGURE 4 | Representative GDF-15 immunostaining of clinical samples from adenocarcinoma patients. (A) A part of the tissue with strong GDF-15 expression (strong [2+]). (B) A part of the tissue with little GDF-15 expression (negative [0]). GDF-15 expression was heterogeneous within the same tissue, with most tissues expressing GDF-15 in some regions. Magnification ×40, scale bar represents 2.5 mm, rabbit anti-human GDF15 polyclonal antibody (ATLAS ANTIBODIES, HPA01119) was used for immunostaining.

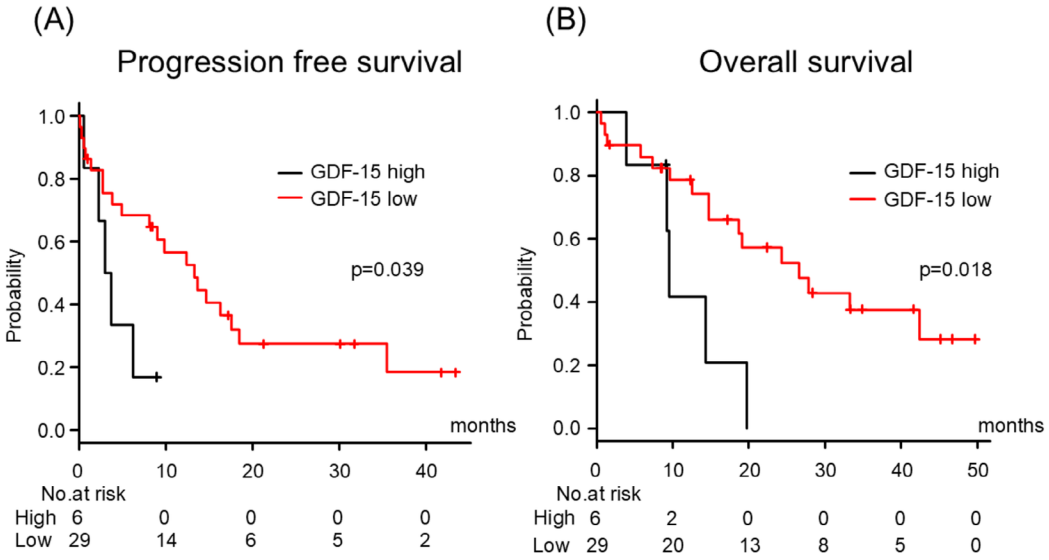


FIGURE 5 | (A) Kaplan–Meier curves of PFS stratified by GDF-15 expression. (B) Kaplan–Meier curves of OS stratified by GDF-15 expression. OS, overall survival; PFS, progression-free survival.

expression in tumor tissues and serum GDF-15 levels, it may not be more effective in patients with high serum GDF-15 levels [24]. In such cases, a tissue GDF-15 scoring system that can assess tumor-specific GDF-15 expression could be a companion diagnostic tool to assess the therapeutic effect of PD-1/PD-L1 inhibitors and GDF-15 neutralizing antibodies. In NSCLC, treatment decisions are made based on the expression of PD-L1 in the tumor tissue; thus, it is feasible to use the scoring system as a companion diagnostic tool [41].

There are several limitations to this study. Primarily, this study represents a single-center, retrospective investigation carried out in Japan, and the sample size was small; therefore, we were unable to exclude bias due to unknown factors. Second, GDF-15 expression is heterogeneous, even within the same tissue; thus, specimens obtained by bronchoscopy were included in this study despite the fact that surgical specimens were originally used. However, we believe that even small biopsy specimens obtained by bronchoscopy can be used to evaluate overall GDF-15 expression using the scoring system since PD-L1 heterogeneously distributed in tumor tissue is considered to represent PD-L1 for the entire tumor during lung cancer therapy [42]. Establishing a scoring system using findings from microtissues may have significant clinical benefits.

5 | Conclusion

GDF-15 expression is heterogeneous in tumor tissues. Furthermore, GDF-15 expression in tumor tissues, graded using

the scoring system, may serve as a predictive indicator of response to PD-1/PD-L1 inhibitors.

Author Contributions

Software: Naoya Nishioka. Validation: Naoya Nishioka and Tateaki Naito. Investigation: Naoya Nishioka and Tateaki Naito. Visualization: Naoya Nishioka. Writing – original draft: Naoya Nishioka and Tateaki Naito. Conceptualization: Naoya Nishioka. Resources: Nobuaki Mamesaya, Haruki Kobayashi, Shota Omori, Ryo Ko, Kazushige Wakuda, Akira Ono, Hirotsugu Kenmotsu, Haruyasu Murakami, and Toshiaki Takahashi. Tissue staining: Takashi Sugino and Koji Muramatsu. Tissue staining evaluation: Takashi Sugino and Koji Muramatsu. Project administration: Tateaki Naito. Writing – review and editing: Tateaki Naito. Methodology: Naoya Nishioka, Tateaki Naito, Shigeki Nishihara, and Hiroki Urashima. Formal analysis: Naoya Nishioka and Tateaki Naito.

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Disclosure

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Ethics Statement

This study was conducted according to the guidelines of the Declaration of Helsinki, 1964, and its later amendments, and was approved by the Institutional Review Board of Shizuoka Cancer Center, Japan (J2021-74) on August 6th, 2021.

Consent

Patient consent was waived due to the retrospective nature of the study.

Conflicts of Interest

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Data Availability Statement

Data are available upon reasonable request to the corresponding author.

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